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EGG-LAYING AND EGG-HATCHING IN *AGABUS BIPUSTULATUS* L., WITH NOTES ON OVIPOSITION IN OTHER SPECIES OF *AGABUS* (COLEOPTERA : DYTISCIDAE)

By DOROTHY J. JACKSON

Manuscript received 3rd September, 1957

(Read 6th November, 1957)

With 13 Text-figures

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I. INTRODUCTION AND ACKNOWLEDGMENTS

In the summer of 1954 I became interested in the egg-laying habits of some of our common water beetles, as I had found the Mymarid *Caraphractus cinctus* Walker (*Polynema natans* Lubbock) to be a common parasite of eggs of Dytiscidae in ponds in Fife (Jackson, 1956a), and I wished to obtain a supply of eggs of various species in order to carry out investigations into the biology of this Fairy Fly. It was essential to obtain eggs of Dytiscidae laid in captivity under

conditions excluding the possibility of previous parasitism and this entailed finding out how and when the different species laid their eggs. While the oviposition of *Dytiscus marginalis* L. (one of the hosts of this Mymarid) has been extensively studied by various entomologists, little appears to have been published regarding the oviposition of some of the common Colymbetines in this country. The present paper is confined to some species of *Agabus*, and it is hoped to publish observations later on *Ilybius fuliginosus* F., a species which has proved to be a useful host for *Caraphractus* during the summer.

The beetles studied were collected in Scotland, principally in Fife, and the notes here recorded are based, firstly, on an examination of the condition of the reproductive organs of specimens collected at various times of the year in order to find out at what season the ovaries become mature, and, secondly, on experiments undertaken to get the beetles to lay in captivity on plants occurring in their natural habitats. In addition, eggs collected on water plants in ponds were reared, and the larvae were identified with the help of Bertrand's work (1928). The position in which the eggs are laid is dependent on the structure of the ovipositor. This is well shown by comparing the ovipositor of an *Agabus* with that of an *Ilybius*, for in the latter genus the ovipositor blades have teeth like a saw and the eggs are embedded in plant tissues. An account is therefore given of the female genitalia in *Agabus bipustulatus* which are compared with the genitalia of the other species dealt with.

I wish to express my gratitude to Dr. M. D. Dunn of the Department of Botany, St. Andrews University, for the invaluable help she has so kindly given me in the identification of the water plants. I am very grateful to Dr. F. I. van Emden and to Mr. J. Balfour-Browne for reading over and discussing the manuscript of this paper. Mr. Balfour-Browne has kindly allowed me to include some of his data on the breeding of *Agabus*, as cited in the notes following.

II. *Agabus bipustulatus* L.

This is our commonest and most widely distributed *Agabus*. It is usually to be found in any stagnant pond at any time of the year.

1. *Time of Oviposition*

In soft, newly emerged beetles the ovaries are extremely small and quite immature. I do not know how long it takes for the ovaries to become fully developed, but specimens which were collected in early August, 1955, in Inverness-shire, and which were already hard (so not just newly emerged) had the ovaries still immature a month later, though the ovaries were larger than in soft specimens. It seems probable that a period of two or three months is required for the full development of the ovaries. Soft specimens have been obtained in Scotland from the end of May till the beginning of August. Balfour-Browne (1950) in his chart (p. 272) records soft specimens from May till September.

From the dissection of many specimens of both sexes throughout several years—the dissections were carried out principally during my investigations into the condition of the flight muscles—it is evident that great individual variation exists in regard to the degree of development of the reproductive organs in specimens collected in any one month; this will be due to the fact that emergence

occurs over a period of several months in the summer, so that the specimens dissected at any one time will often be of different ages. In the majority of specimens, however, the ovaries are fully developed from the end of August (in Fife) throughout the winter up till May, while in most females collected in July the ovaries are extremely small. In Scotland the species appears to lay principally in autumn, winter and spring, but Mr. Balfour-Browne has found first-stage larvae of this *Agabus* in the field in all months of the year. He has had females ovipositing not only in their second but also in their third year. In Germany, Blunck (1921) records that the breeding time of this species extends from autumn till spring. Bertrand (1928 : 308) states that, while most of the *Agabus* in France lay from March to June, *Agabus bipustulatus* lays repeatedly during the year, principally in autumn, winter and early spring.

The habit of *Agabus bipustulatus* of laying during the winter months may be one of the factors contributing in two ways towards the abundance of this beetle. Firstly, its eggs are readily parasitised by *Caraphractus cinctus*, but this Mymarid is inactive during the winter months, hibernating in host eggs principally in the prepupal state, so that all the eggs laid by the beetle at this season will escape parasitism. Secondly, the larvae hatching from eggs of *A. bipustulatus* laid late in the year will not experience drought, the entire life cycle being completed in cool weather. Eggs laid from the end of September till mid-December, when reared in an unheated room, produced imagines from the middle to the end of May. These beetles would doubtless be ready for oviposition by the following autumn.

2. Fertility

The fertility of *A. bipustulatus* during the winter months is well illustrated by a single female which I kept under observation in an unheated north room. She laid about 350 eggs from the end of September until the beginning of May. The actual number was probably greater because the beetles readily eat their eggs and a male was kept with her till 21st November. After the removal of the male, egg production increased, 322 eggs being obtained, of which 242 were laid before the end of March. Even those laid just before the death of the female in early May were fertile. An even higher record of egg-laying was obtained from another female collected in the middle of August, 1956, which was kept alone in a tumbler of water and fed plentifully with *Chironomus* larvae, Lumbriculids and crushed *Asellus* and *Gammarus*, and latterly with *Tubifex* worms. From 20th August till 2nd July she laid 1693 eggs, and this number does not include the eggs she had deposited and eaten, the remains of which were often found in the foliage. During June fewer eggs were laid, and although oviposition ceased during July, by the end of August she had laid 11 more eggs. Throughout this long period—from August, 1956, up to the middle of June, 1957—almost every egg she laid developed normally, unless parasitised by the Mymarid which only develops on fertile eggs. Only amongst the last 18 eggs laid were the majority infertile. By this time the female had been isolated for 12 months showing the great viability of the spermatozoa she had received before capture. On 1st September I placed a male with this female for 19 days, and she laid, until her death in early November, 93 more eggs, nearly all fertile, bringing her total egg production in captivity up to 1797. From other *Agabus* (*paludosus* F.

and *nebulosus* Forst.), collected in the autumn, I have occasionally obtained infertile eggs in spring; such eggs look normal when first laid but later become blotchy and discoloured. They often develop a fungus which Mrs. Balfour-Browne has kindly identified as a *Saprolegnia*. She informs me that this fungus generally attacks only damaged and decayed tissue. Only an occasional egg of the prolific female of *A. bipustulatus* developed fungus.

According to Blunck (1914: 87-88) the spermatozoa in the female *Dytiscus* can remain alive for 9 months, but I have had females of *D. marginalis* laying fertile eggs 11 months after isolation from males. The highest number of eggs which Blunck records from one individual of *Dytiscus* was only 203, laid within a fortnight (Blunck, 1913b: 163). I have had 24 eggs laid by one female of *Agabus bipustulatus* within five hours. This followed the placing of fresh sphagnum moss in the jar. I have found that the presence of fresh plants of a type acceptable for oviposition stimulates the deposition of eggs; in the absence of suitable plants my observations indicate that the females are able to withhold most of their eggs, but may deposit a few on the glass of the container. When eggs are being produced regularly by a mature female much food is consumed (24 Chironomid larvae, 8-10 mm. long, are disposed of by one female in 48 hours), but females that are not laying eat much less. It would appear that in unsuitable breeding conditions the females cease to lay and adjust their feeding accordingly.

3. Literature on Ovarian Development in Dytiscidae

No information appears to have been published on ovarian development in an *Agabus* but, of *Dytiscus marginalis* L., Korschelt (1924, 2: 804) states that the majority of the beetles emerge in August and September and the first eggs are laid in March (p. 358). He records (p. 359) that this species may live up to four years, and, in captivity, the beetles have been observed to lay again in their second year, the ovaries after the first laying period entering into a resting condition. Joly (1945) observed that laying in *Dytiscus* is not influenced in any way by the external environment. If, however, the temperature is low or the feeding insufficient, the beetle reabsorbs its adipose tissue and, if the beetle survives, the ovaries will develop at the normal time.

4. The Female Genitalia

In the following notes the general structure of the female genital armature is briefly described, but no study has been made of the muscles which control the functioning of the genital segments. In the present investigation the interest lies in the character of the ovipositor blades which deposit the eggs; and the basal sclerites which support these blades and render their function possible are therefore described. The whole of the laying apparatus, when not in use, is normally withdrawn telescopically into the apex of the abdomen, where it is concealed above by the eighth tergum, and below by the seventh sternum. It has been noticed, however, that females often die with the ovipositor and its associated sclerites protruded, and in a newly dead beetle these parts can be forced out by pressure over the anterior part of the elytra, but this latter method may lead to distortion. It was used, however, in examining material and once the genitalia were sufficiently protruded the beetle was

plunged into absolute alcohol. Good results were also obtained by soaking the posterior abdominal segments in dilute caustic potash, dehydrating and examining in clove oil or alcohol.

Considerable confusion occurs in the literature regarding the names applied to the different parts. The principal work in which the genital armature of an *Agabus* has been described is that of Böving (1913), but Blunck (1913a), Guignot (1933), and Balfour-Browne (1950) have also published some data. It will be convenient therefore to give the names used by these writers for the principal sclerites of the laying apparatus. Commencing with the proximal sclerites they have been named as follows:—

Böving (1913)	Cloaca-valve or pleurosternite VIII	Fork with arm and summit	Genital valve	Vulvar sclerite.
Blunck (1913a)	.	Dreieckige Chitinstück	Seitenspange	.
Guignot (1933 : 60, 552)	Sternite VIII or valve génitale	Paravalvifère and proctigère	Lame de l'ovi- scape	Vulvosclerite.
Balfour-Browne (1950 : 7)	Eighth sternum	Valvifer and supra-anal sclerite or epiproct	Genital valve	Vulval sclerite.
Jackson	Sternite 8	Valvifer	Blade of ovipositor	Vulval sclerite.

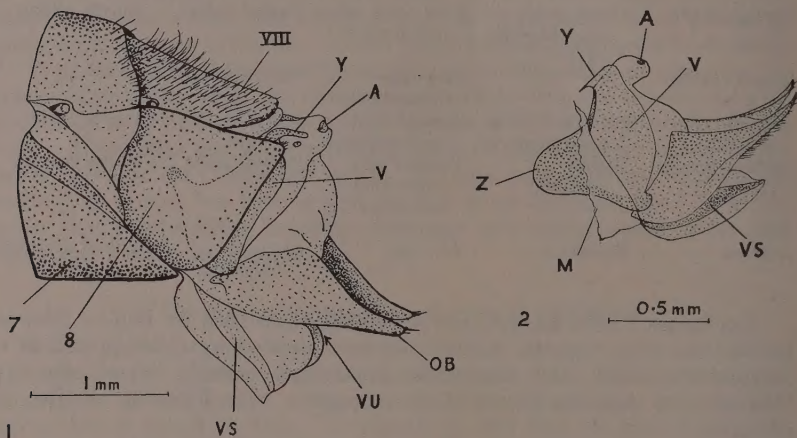
The names I have adopted are principally those used by Balfour-Browne, but, as the term "genital valve" has been applied by different writers to different structures, and since these appendages actually deposit the egg, I have called them the blades of the ovipositor. The following sclerites are all paired.

Sternite 8.—These two plates form the sternum of the eighth segment and they are entirely concealed within the abdomen except during oviposition. They are strongly sclerotic and are united ventrally by membrane. Since no pleurite can be distinguished in segment eight, Böving has termed them pleurosternites.

Valvifer.—I have applied this term to the whole of the sclerotic area lying on each side of the genital segment. This sclerite is largely concealed beneath the eighth sternite, and the outline of the hidden part is indicated in figure 1 with broken lines. Dorsally the valvifer extends towards the anus. The anus opens on a fleshy segment, the "anal segment" of Korschelt (1923, 1 : 72), behind the eighth tergum, and he designates the anal plates of this last segment as "IX" in his figure of the abdomen of *Dytiscus*. The valvifer in *Agabus bipustulatus* would appear to be homologous with the sternite of the ninth segment, and the upper portion of this sclerite, which is produced into a curved process, will be the equivalent of the epiproct or anal plate. Within this curved process, on each side of the body, is the opening of the duct of the reservoir of the anal gland. A milky-white fluid is discharged from either orifice on pressing the corresponding reservoir of a newly killed beetle. The valvifer is shaped in this species as a comparatively narrow upright plate, and it is produced anteriorly into a deeply sclerotic internal strut or apodeme which penetrates inwards and forwards beneath the segmental membrane connecting

the valvifer with the eighth sternum. The lower posterior angle of the valvifer articulates with the base of the ovipositor plate. In *A. bipustulatus* the valvifer thus forms a well defined plate on each side, and the plates are not continuous dorsally. In *Agabus undulatus* Schr., which Böving figures, the dorsal area of the valvifer is only faintly sclerotic, so that the upper termination of the valvifers is not clearly defined, and this will explain why he referred to these sclerites as an unpaired structure, or a fork with summit and two arms.

The ovipositor blade.—Viewed laterally, each ovipositor blade is wide at the base and thence tapers towards the tip or apex which is more rounded than



FIGS. 1-2.—(1) *Agabus bipustulatus* L. Posterior segments of female with ovipositor extruded. (2) *A. chalconatus* Panz. Ovipositor and anal segment. The eighth sternite has been removed to expose the valvifer. (For explanation of lettering see p.79.)

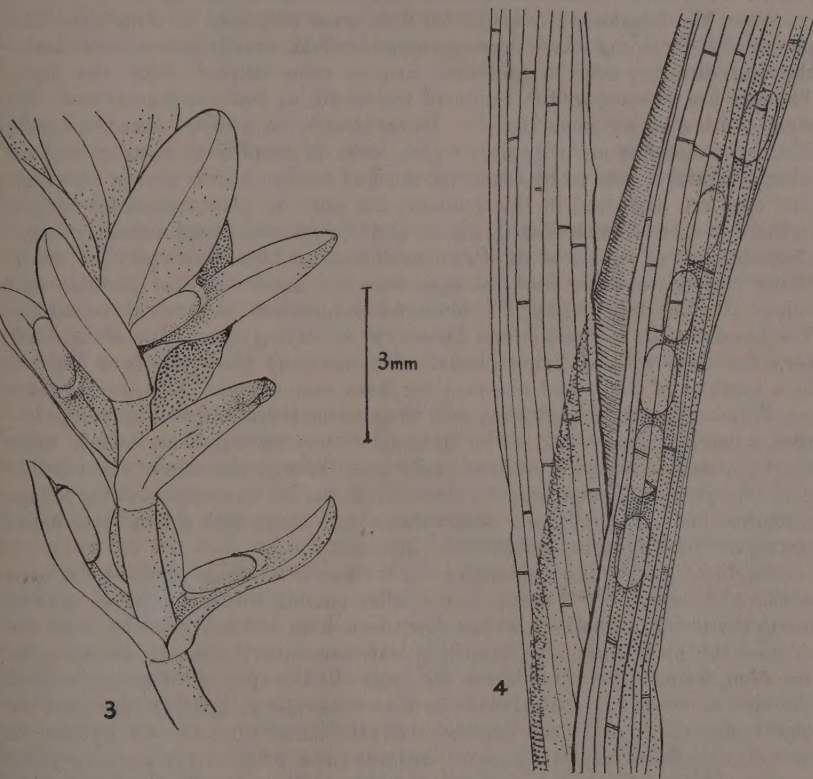
acute (fig. 1, OB). The inner surfaces of the two blades face each other so that they will have a forcep-like action in guiding the egg into position. Each blade is provided at the apex with what appears to be—under low magnification—a single stout bristle, but it is in reality a tuft of about a dozen closely placed parallel bristles with blunt ends. In addition, many shorter bristles of the same type are situated at the base of the tuft and over the distal part of the blades, while much shorter and thicker cone-shaped bristles are densely crowded near the tip. These various bristles will, with little doubt, be sensory bristles enabling the beetle to locate the right place to put her eggs. Between the blades of the ovipositor is the vulva, the external opening of the vagina. The ovipositor blades are feebly sclerotic, soft and pliable and they are unsuited to piercing healthy plant tissue.

The vulval sclerite.—This is a weakly sclerotic plate, wedge-shaped and tapering distally. One is situated on each side of the membrane lying below the vulva. The ventral membrane between these sclerites is covered with an intricate arrangement of flat-lying, overlapping bristles, with bifurcating bases,

which will strengthen the membrane below the vulva where it rests against the plant tissue during oviposition.

5. Oviposition

Agabus bipustulatus lays its eggs on a variety of water plants, always under water. In captivity females have laid on *Elodea canadensis* Michx. (fig. 3), on *Lemma*, on *Veronica beccabunga* Linn. and very readily on sphagnum moss.



FIGS. 3-4.—*Agabus bipustulatus*. (3) Four eggs on leaves of *Elodea canadensis*. (4) Six eggs laid under sheathing leaf of *Glyceria plicata* Fries.

The broad-leaved varieties of Sphagnum are preferred, and principally the green shoots, each egg being fitted into the hollow of a leaf (fig. 6), always with the anterior pole towards the apex of the leaf. Occasionally an egg may be placed along the sphagnum stem and they may be laid in this manner on the fine-leaved Sphagnums (*S. flumulosum-acutifolium*) in which the leaves are too small to support an egg. The broad-leaved Sphagnum has proved to be the most useful plant on which to collect the eggs of this and of other *Agabus*

species for use with the Mymarid parasite, for, if the moss is gathered from an area where no water is lying, it is free of water beetle eggs, and can be kept fresh for months in water; moreover the eggs are readily visible. The beetles affix the eggs to the leaves by a transparent gelatinous cement which may partly surround the egg, sometimes filling up the apex of the sphagnum leaf (fig. 6). On the other plants mentioned the eggs are scattered about and not laid in a regular manner (fig. 3). On the green shoots of the moss *Fontinalis antipyretica* Hedw. the eggs are inserted within the narrow folded leaves (fig. 5).

When the female is provided with flote grass (*Glyceria*) or other grass-like plants with sheathing leaves numerous eggs are laid, usually in even rows under the leaf sheaths, even when these are in close contact with the stem. The eggs are fastened to the inside of the sheathing leaf—or sometimes to the stem—with a little cement (fig. 4). It has already been shown that the female of this species has an ovipositor which looks ill-adapted to cutting, and no wound whatever was to be found on the leaf surface above or near the eggs. The eggs are deposited in this manner, not only on plants standing upright in the water, but also on floating pieces, and 26 eggs were found under the tough sheathing leaf of a green stem of *Juncus articulatus* Linn. floating in the water. Being curious to know how the eggs were laid under the leaf sheaths, and using the following method, I observed oviposition on several occasions. The female to be observed (one known to be laying) was fed well and left for a few days without living plants. She was then given shoots of *Glyceria* in a tumbler of water and observations were commenced. Even under these conditions eggs were deposited at such irregular intervals and with such rapidity that a constant watch had to be maintained over several hours. Only when she happened to adopt a position easily seen through the side of the tumbler could the process of oviposition be observed, as the least movement of the foliage disturbed her. The following observations were made with a hand lens under strong artificial light and noted with quick sketches.

All the time the beetle is under water there is a bubble of air at the apex of the abdomen. When about to lay, after coming to the surface to take in air in the usual fashion, she swims down to a stem and moves along it till she reaches the place where the sheathing leaf surrounds the stem. Standing on the stem, facing either up or down, she protrudes the apex of the abdomen from the elytra, curving it ventralwards so that the sixth to eighth terga—and the eighth sternum (fig. 1)—are exposed. At the same time the air bubble—in contact with the subelytral air space—spreads out as a flat silvery dorsal covering to these tergites, somewhat obscuring the view. The ovipositor is then quickly extruded and thrust in between the edge of the sheathing leaf and the stem, so that it lies at right angles to the long axis of the beetle's body, *i.e.* in a horizontal position when the stem is vertical. The yellowish ovipositor probes about under the leaf sheath up and down, it pauses and then an egg suddenly appears from the vulva above the apex of the ovipositor if the female is facing downwards and below it when she is facing upwards. The egg is thus deposited parallel with the long axis of the beetle's body. She then moves a trifle along the stem, sliding the ovipositor along at the same time inside the sheathing leaf. She stops and after another short pause another egg is emitted exactly in line with the first one; sometimes the two blades of the ovipositor can be seen moving together at the tip as though pushing off the egg. These actions

are repeated until a line of eggs is deposited in a vertical row behind the beetle. I have seen seven eggs laid thus in barely five minutes. While a single row is being laid the ovipositor is not withdrawn, but merely slides along fully extended inside the sheathing leaf. There is no cutting done and the weakly developed vulval sclerite probably serves merely to protect the side of the vulval lobe during laying. Sometimes, especially in detached sheathing leaves, the eggs are laid in several parallel short rows and each row will represent a separate insertion of the ovipositor at a different depth.

What struck me most was the extent to which the female genitalia could be extruded horizontally *i.e.* towards the inner part of the leaf. The figures given by Böving (1913) of *Agabus abbreviatus* F. (*undulatus* Schr.) only partly correspond to what I have seen in the living female of *A. bipustulatus*. I saw no bulging ventral portion of intersegmental cuticle below the ovipositor, and Böving's figure is doubtless the result of pressing out the ovipositor, for this membrane shows to some extent in the ovipositor I have figured (fig. 1) which was also forced out.

One curious lapse in the orderly method of oviposition was observed when a female that had laid three eggs under a sheathing leaf of *Glyceria* was seen within half an hour of laying to be tearing at the leaf with her mandibles just above where the eggs were laid. She succeeded in tearing the leaf into pieces and then she ate the eggs! Within 20 minutes she laid four more eggs and later attacked the leaf and ate them also, yet she had been fed daily on *Chironomus* larvae and on small worms.

I have much experimental evidence to show that laying under the sheathing leaves of grass is the preferred site for oviposition of *Agabus bipustulatus* for, when females were placed with *Glyceria*, Sphagnum and other plants, in all cases numbers of eggs were laid on the *Glyceria* and very few on the other plants. Probably all plants with sheathing leaves will be used for laying, provided the leaves are not too stiff, and do not grip the stem too tightly. In the case of a coarse grass with a stiff sheathing leaf (it was probably a species of *Agropyrum* that was growing in water) the female only laid in a part of a leaf sheath which was loosened from the leaf, and, in a detached leaf which had folded into a stiff cylinder, 19 eggs were deposited. The older detached sheathing leaves of *Glyceria*, a softer grass, in which the edges of the leaf overlap to form a tube, are frequently chosen for oviposition. In all these cases the female had pushed her ovipositor in through the opening and no cutting was done. Females have laid also under the sheathing leaves of *Carex otrubae* Popd. and *Juncus squarrosus* L., and under the sheathing base of the leaf of *Ranunculus flammula* L.

This method of oviposition—under sheathing leaves—will protect the eggs in several ways. Firstly, the eggs being concealed from view will escape the notice of some predators although, as has been shown, it will not always protect them from attack by the beetles themselves! Secondly, although the females of *Caraphractus* quickly perceive the eggs and attempt to lay in them, I have observed that they have great difficulty in penetrating with their ovipositors the living sheathing leaf of *Glyceria*; instead, whenever a chink allows, they creep under the sheathing leaf and lay directly in the eggs. So it is likely that eggs well enclosed by tightly encircling leaves will have some protection even against the ovipositor of *Caraphractus*. But perhaps the greatest advantage in this method of laying is that eggs so deposited have a better chance of sur-

viving during drought than eggs exposed on leaves. This is shown by the following experiment. Stems of *Glyceria*, containing eggs of *A. bipustulatus* from one to three days old, laid under the sheathing leaves, were so cut that when placed in 3 inch by 1 inch glass tubes containing only half an inch of water, the eggs were situated from $1\frac{1}{4}$ to 2 inches above the level of the water, while the cut end of the stalk rested in the water. The tubes were kept in my laboratory and covered with muslin tops. As controls, eggs laid at the same time were kept in water in the same room and these hatched in from 14 to 18 days. Once eggs of *Agabus* dry up they die (on account of the fragile character of the chorion which gives no protection against drought, as will be shown later) but the eggs in my experiments which were lodged under the sheathing leaves well above the water, remained healthy provided they were in contact with both the leaf sheath and the stem. The lowest eggs in contact with leaf sheath and stem were found to be surrounded with moisture, but all eggs on the leaf sheath alone, or on the stem alone, dried up and perished; there had to be a close contact between leaf and stem for survival out of water. Two larvae duly hatched from eggs which had been from 14 to 18 days in a shoot above the water, and the larvae had descended to the water in the foot of the tube. Sometimes, in the eggs so tested, hatching was delayed for some days, but if the eggs were placed in water, the larvae quickly hatched.

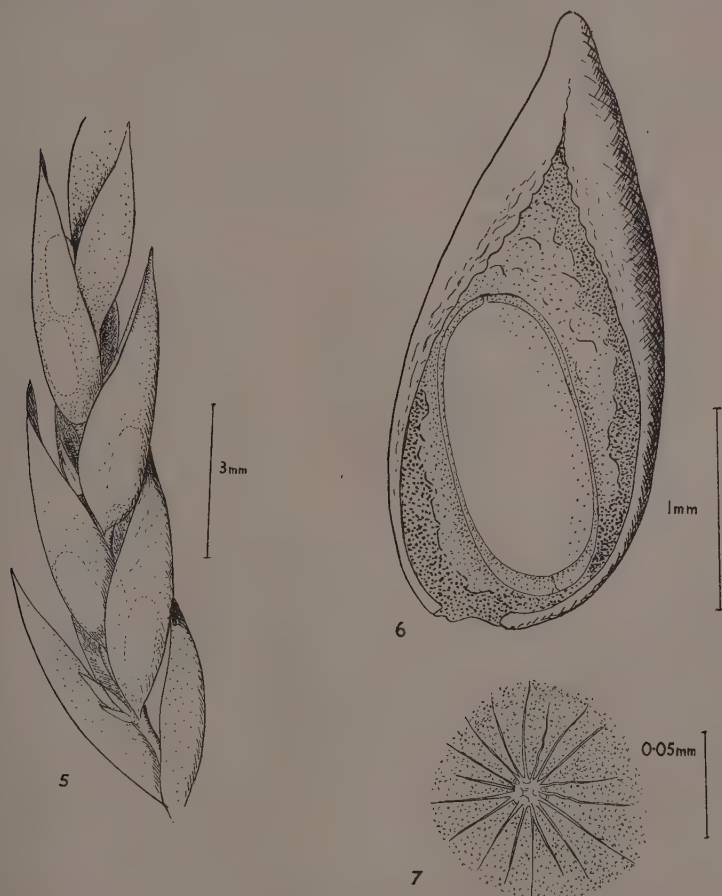
The fact that the eggs can survive out of water when laid within the sheathing leaves of grasses must assist in the survival of many individuals hatching in the warmer weather during a time of partial drought, whereas eggs laid exposed on the leaves of water plants would perish. This would apply especially to a species like the present one which so often frequents shallow grassy ponds. The female of *A. bipustulatus*, though without a cutting ovipositor, is thus able to place her eggs in much the same favourable position for development as the females of *Dytiscus* and *Ilybius* which embed their eggs in living plant tissue, and Blunck (1914 : 99) states that, in the eggs of *Dytiscus*, the embryos develop normally even if, by the growth of the plant, they are carried above the water. My observations on the laying of captive beetles are confirmed by observations on collected eggs. These have been found under the sheathing leaves of *Juncus articulatus* L. along with many eggs of *Agabus sturmi*. The eggs were kept until they hatched, and the first instar of the two species is readily distinguishable once the full coloration is attained, *A. bipustulatus* having a uniform dark greyish brown head, and *A. sturmi* a light brown, variegated head. Eggs of both species have also been found within the brown withered leaves of this *Juncus*, but such decayed leaves are quite soft and so will be easy for the ovipositor to penetrate.

Blunck (1921) has recorded the oviposition of *Agabus bipustulatus* on both living and dead leaves of *Myriophyllum* and *Myosotis palustris* and he notes that the beetles prefer to lay on fresh green shoots and put their eggs between the youngest leaves without wounding the tissues. In *A. undulatus* Schr. he states (1913a) that the eggs are laid in the fresh green buds of water plants such as *Elodea canadensis*, hidden in the axils of the leaves. He considers the ovipositor of *A. undulatus* to be quite unsuited for cutting activities. Wesenberg-Lund (1912 : 16) describes finding numbers of eggs of *Agabus* in holes bored by the ovipositor in stems of *Ranunculus lingua* and he figures such a stem (1943 : 303, fig. 264). He also records that one *Agabus* species lays its eggs within

the leaves of *Hypnum*, splitting the leaves; but unfortunately he does not, in either instance, name the species of *Agabus*. Since *A. bipustulatus* lays between the closely folded leaves of the moss *Fontinalis antipyretica* (fig. 5) so that some eggs may appear, from a cursory examination, to be within the leaf, perhaps this may explain Wesenberg-Lund's observation regarding *Agabus* eggs in *Hypnum* leaves.

6. The Egg

The newly laid egg (fig. 6) is of a pale translucent yellowish colour. It is soft and easily indented. Measured when under six hours laid the eggs show



FIGS. 5-7.—*A. bipustulatus*. (5) Five eggs laid within folded leaves of *Fontinalis antipyretica* Hedw. (6) Egg under 24 hours laid in leaf of *Sphagnum*; the adhesive cement has been removed from the surface but is shown *in situ* around the egg and extending towards the apex of the leaf. (7) Surface view of micropylar area of egg. Drawn from the chorion, slipped off the egg, within 24 hours of laying.

a good deal of variation in size, from 1.36 by 0.63 mm. to 1.53 by 0.68 mm. For the first few days after laying the egg remains the same size and by the third day it has become firmer. After this it gradually increases a little in size, most noticeably in width. Measurements have been made of eggs within six hours of laying and again on the day of hatching. I have submitted these measurements to Dr. van Emden, who has most kindly calculated the increase in size. He finds that, when ready to hatch, the egg has almost twice the volume it had shortly after being deposited, or, if the contents of the vitelline membrane alone are calculated for the young egg (for, as is noted below, the vitelline membrane does not reach to the chorion in the newly laid egg) the increase in size is 2.6 times. When of full size the egg is quite firm. As already mentioned in the preceding section, the egg is affixed to the plant tissue by a colourless transparent gelatinous cement which largely surrounds the egg. As the egg develops, the gelatinous layer hardens and becomes a pale amber colour or even brown. It may break away over the centre of the egg, but it usually adheres closely to the ends and along the side of attachment. This adhesive layer shows up well if the leaf on which the egg rests is stained. With eosin (a 2.5 per cent. aqueous solution was used) it stains bright pink, while with methylene blue it becomes a very pale blue in vivid contrast to the bluish-purple colour of the stained sphagnum leaf.

The newly laid egg shows a double contour, a loosely fitting outer membrane, the chorion, and an inner, the vitelline membrane, surrounding the egg contents. At the anterior pole of the egg a round whitish spot can be seen in the chorion with low magnification. This is the micropylar area, a stellate structure (fig. 7) with radial processes varying in number from 17 to 20. Beneath the chorion a translucent connection leads from the micropylar area to the vitelline membrane and so to the egg contents. At the posterior pole of the egg a somewhat similar structure occurs, but smaller and without such well developed radiating processes. It also, in the newly laid egg, shows a connection with the vitelline membrane. It may possibly be a second micropylar area, for Luginbill (1953) records a micropyle at both ends of the egg in the *Lamellicorn*, *Phyllophaga*.

I have frequently noticed when removing eggs of *Agabus bipustulatus* at various stages of development from leaves, that the chorion comes off easily. Moreover in a number of cases the egg, deprived of its chorion and enclosed only by the vitelline membrane, continued to develop normally and the larva duly hatched. This occurred in eggs from which the chorion had been removed within 24 hours of laying and up to four days after. The "naked" egg floats at once to the surface and has to be pushed into the water to allow of development. A similar development of eggs deprived of the chorion is recorded by Weismann (1863 : 160) in *Musca vomitoria* (now *Calliphora vomitoria* L.). In order to study the embryology of this fly he removed the opaque chorion from the egg, exposing the vitelline membrane, and some of the eggs so treated hatched.

Under normal conditions I have found that the chorion of the *Agabus* egg persists. It can be seen in eggs nearly ready to hatch and also in the hatched egg if examined in transmitted light. It is easily located at the head of the egg by the micropylar area and it can be traced round the side of the egg (when this is not attached to the leaf) to the similar structure at the posterior pole. It appears in optical section as a thin refractile layer above the thicker and more homogenous vitelline membrane, and, in surface view, it has a slightly granular

appearance. It is, however, a very fragile membrane and if the egg is removed from the substratum it readily tears or comes off altogether. It lies at first loosely round the egg (so loosely that any pressure on the leaf causes it to crease), but, as the egg swells, the vitelline membrane comes to lie closer to the chorion so that the latter only appears as a separate membrane at the poles. The positions of the two membranes may again fluctuate but latterly they are in close contact.

The fragility of the chorion in this species is probably the explanation of the statement made by Blunck (1921) that, as the egg increases in size, the chorion is no longer able to resist the growing internal pressure and breaks, so that the vitelline membrane ("Dotterhaut") comes to lie on the outside of the serosa ("Eihüle"). Possibly the chorion in the eggs he observed had been damaged by removal from a leaf. He deals with this matter in much more detail in the eggs of *Dytiscus* (Blunck, 1914) and he notes that as the *Dytiscus* eggs increase in size they become tense and elastic. The change in consistency of the vitelline membrane he finds is effected by the serosa strengthening the membrane through chitin-like secretions (Blunck, 1914: 86). He states that in the course of the embryonic development of the *Dytiscus* egg the chorion is entirely removed from the vitelline membrane, even the connection at the micropyle being broken, so that the fully developed embryo is only surrounded by the vitelline membrane (Blunck, 1916). I have examined many eggs of *Dytiscus* both before and after hatching, and I find that the chorion continues to enclose the greater part of the egg to the end, but some days before hatching it normally splits lengthwise over the midventral and the mid-dorsal line so that it becomes divided into two equal halves which are still closely applied to the vitelline membrane; at other times the splits in the chorion are more irregular. The anterior pole of the egg, surrounded by the vitelline membrane alone, is always exposed from the chorion before hatching, and the connection between the micropylar area on the chorion and the vitelline membrane is broken. The chorion, however, is so transparent that it is readily overlooked in the egg about to hatch unless stained with methylene blue.

Blunck (1914: 90, 91) observed that in the egg of *Acilius sulcatus* L. the space between the chorion and the vitelline membrane increases when the air is saturated with moisture, but disappears in dry air. If the egg of *Agabus bipustulatus* is placed on damp blotting paper and allowed to dry, within an hour the chorion has shrunk closely to the egg. In *Acilius*, Blunck found that the pressure of the fluid between the chorion and the vitelline membrane is so great that by piercing one of the poles of the egg, the egg comes out of the chorion quickly and with vehemence, just as I have observed in *Agabus*.

It is curious that Blunck should describe the chorion of the egg of *A. bipustulatus* as turning brown during development. I have found it to remain almost colourless throughout development, only the palest brown tinge being discernible latterly under high magnification, but the external adhesive layer deepens considerably in colour.

It is clear that the chorion of the egg of *Agabus bipustulatus* (and apparently also of other Dytiscidae) is of very fragile character. Since the egg is laid in water the chorion will not be required to conserve moisture, and the thinness of this membrane will enable the water to pass more easily into the egg to allow of the embryo's development. In eggs developing in eosin or in methylene blue,

these stains pass into the gut contents of the larva, so the various membranes must be readily permeable.

The fragile character of the chorion and the major role played by the vitelline membrane in protecting the growing embryo is undoubtedly associated with the aquatic environment. Secreted by the follicle cells of the ovariole, the chorion has no doubt served its purpose once the egg is fertilised and then laid, and the vitelline membrane finally develops to form the principal part of the egg shell, perhaps strengthened by secretions from the serosa, and, should the chorion become detached, the larva of *Agabus* can still, in some circumstances, complete its development.

7. Hatching

During the winter in a warm room the eggs usually hatched in about a fortnight. They were kept in small dishes of water and their development and hatching were observed with a dissecting microscope. For more detailed examination with a compound microscope the eggs were mounted in water in an excavated slide. Some of the mounted specimens emerged normally, especially if just ready to hatch at the time of mounting, but the development of other mounted specimens was delayed for days, and yet if removed to a dish of water they revived and even hatched. It is believed that lack of dissolved oxygen in the water of the slide cavity accounted for this arrest in development. Even when eggs were kept in dishes of water their development was variable, so that it is impossible to give a precise time-table of the changes leading up to hatching, but it will be convenient to describe briefly the appearance of the developing larva at three stages.

(a) Embryo about a Week before Hatching

Eight days before hatching the shape and position of the future larva is evident. The embryo does not nearly fill the egg, and the legs are short. Pleuropodia are distinct on the first abdominal segment and are similar to those figured by Korschelt (1924) in *Dytiscus*, and by Balfour-Browne (1922) in *Pelobius tardus* Herbst (now *Hygrobia hermanni* F.). The ocelli (in two groups of six) are already pale red. A nucleated membrane, doubtless the serosa, can be seen encircling the embryo but, later on, this membrane could not be distinguished. A week before hatching the embryonic cuticle is formed. It shows most clearly round the legs and mouth parts, appearing as a hyaline membrane over the epidermis, by which it is secreted. Two days later the embryonic cuticle has become further separated from the epidermis and shows very distinctly as a uniform unsegmented cuticle surrounding the embryo and loosely investing the antennae, palps, mandibles and legs in individual sheaths (Jackson, 1957). The two cerci at this stage of development are fully extended within a single tapering sac of cuticle, a prolongation of the embryonic cuticle surrounding the body. Several days before hatching the embryo larva becomes capable of moving its body, head, legs and mouth parts, and sometimes the head may be bent further down towards the centre of the egg than in the normal position shown in figure 8.

(b) Embryo Larva within a Few Hours of Hatching

The larva has increased greatly in size and fills the egg closely. It is still a pale translucent yellowish colour and the gut is filled with yellow spheres,

the remains of the yolk. The larva lies in a curve so that the end of the abdomen is directed forwards. The larva now fits tightly inside the embryonic cuticle so that the head and the various appendages show numerous creases (fig. 8A). The cerci, being now much larger, are bent near their base into an S-like curve, so that in the full grown embryo larva the proximal and subterminal groups of bristles lie together and curve round the neck of the larva. In all the specimens

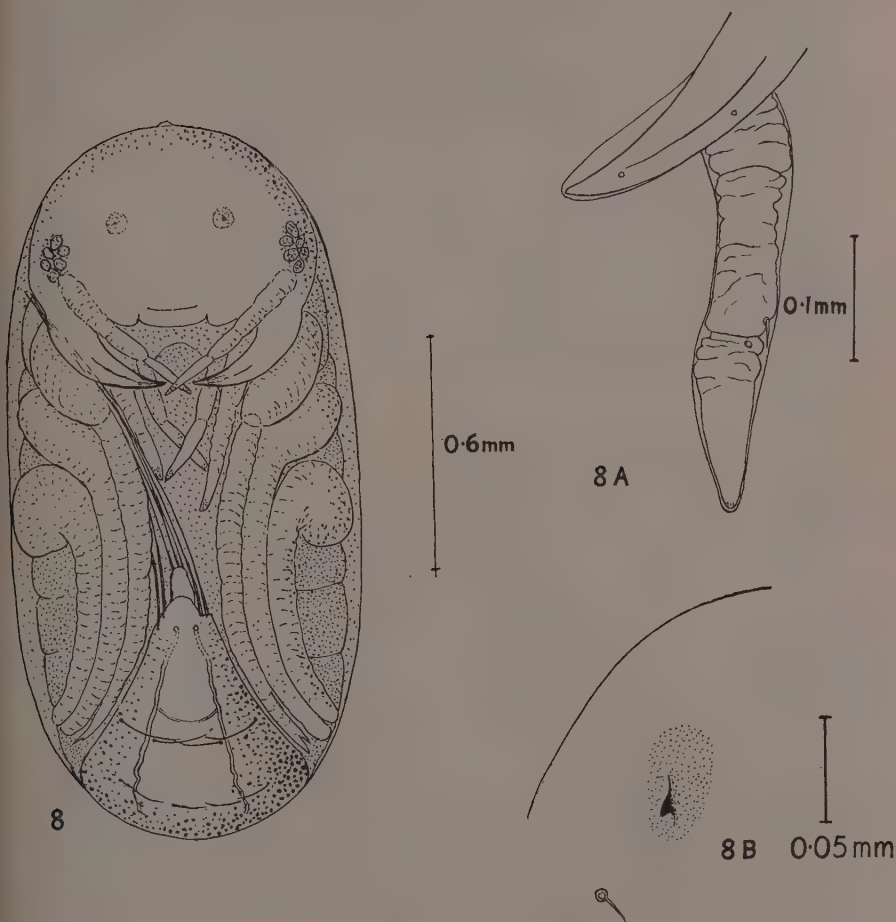


FIG. 8.—*Agabus bipustulatus*. Egg with larva nearly ready to hatch. The embryonic cuticle which covers body and appendages has not been shown and only the conspicuous air-filled tracheae on the posterior segments are delineated. The bristles from the cerci may be seen passing forwards beneath the right mandible to the side of the head. (A) Maxillary palp and apex of mandible of larva at same stage of development as in figure 8, showing the embryonic cuticle and the crinkled segments of the palp, (B) Lateral view of "egg-burster" of right side with apex of spine facing downwards, from living larva of *A. bipustulatus*, nearly ready to hatch.

I have observed these dark cercal bristles lie on the right side of the head (fig. 8). The other body bristles are by this time dark and conspicuous, flattened against the body by the embryonic cuticle; the long bristles of the posterior segments of the abdomen overlap each other so as to lie transversely across each segment. The ocelli are dark red and the mandibles are brown. The two egg-bursters are now very conspicuous, consisting each of a short black spine arising from a pigmented spot on the frons. The tips of antennae and palps have become dark. The tracheae usually fill with air from three to nine hours before hatching. The gas appears quite suddenly, being first noticeable in the longitudinal trunks and then showing in the other branches which are much crinkled. An hour or two before hatching the uniform translucent appearance of the larva fades so that it becomes whitish and dry-looking, due no doubt to the larva swallowing the fluid within the egg. This change is preceded by and concurrent with peristaltic movements of the gut which cause the yolk spheres to move forwards and backwards. Spasmodic pulsations may also be seen within the head in the pharynx below the egg-bursters, and these movements do not always coincide with the movements of the gut contents. In lateral view the pharyngeal muscles, extending from the pharynx to the back of the head, show prominently, and the pulsating of the dorsal vessel is easily seen through the transparent egg-shell.

(c) Larva Just Before Hatching

When hatching is imminent there is usually a violent agitation of the gut contents in the anterior region as though the yolk were being churned up and driven forwards. At the same time the palpitating movements of the pharynx are very evident, but, apart from this internal commotion, the larva is unusually still before the sudden raising of the head which effects its liberation. There is no rubbing of the head up and down within the egg-shell such as Balfour-Browne (1913) observed in *Dytiscus* before hatching. The *Dytiscus* larva, however, emerges from a much smaller opening confined to the anterior pole of the long egg, whereas in *Agabus* the larva emerges from a long split down the egg. I noticed that in the egg of *A. bipustulatus* prior to hatching and during the more violent movements of the pharynx, the egg-bursters were sometimes pushed outward and then withdrawn, but they were never seen to make any cut in the egg-shell. Possibly they may serve finally to cut the embryonic cuticle which tightly invests the head and the entire larva before emergence, for, during a normal hatching, the anterior segments of the larva come out first and are free of the cuticle which has slipped backwards and still encircles the hinder part of the body.

Just before hatching at one moment the larva is lying quietly—there may be a slight motion of the head or a clenching of the mandibles—and the next moment the head jerks upwards and outwards through the egg-shell and the larva slithers out of the egg through a longitudinal slit down the front. Hatching is accomplished with such speed that it is impossible to see whether or not the egg-bursters come into action. The vacated egg-shell shows a single slit extending usually from across the anterior pole, or else from beside the micropyle, almost to the posterior pole, and I have failed to find any transverse slit at the level of the egg-bursters.

8. Delayed Hatching

Some larvae mounted on excavated slides became quiescent when only half-emerged from the egg. In these larvae the embryonic cuticle still surrounded all parts of the larva which protruded from the egg, though the shell of the egg, consisting of chorion and vitelline membrane, was split in the usual way. Each egg-burster spine lies in a slight depression of the frons, and the embryonic cuticle covering the head could be seen clearly in profile overlying the spine in one half-emerged larva. In another instance a larva, appearing ready to emerge when mounted, was lifeless a week later and the gas in the tracheae had disappeared, but on the eighth day the larva emerged backwards, the head still being surrounded by the shell. In all these cases a partial hatching had been effected without the spines coming into use. That the frontal spines are quite ineffective in opening the shell during up and down movements of the head is shown by the following observation. One mounted larva, appearing nearly ready to hatch, was seen to make continuous movements of the head, sliding it up and down within the egg-shell for nearly two hours, but no break occurred. The egg-bursters showed clearly but they never came in contact with the inside of the egg-shell, being covered by the embryonic cuticle. The following morning this same larva was motionless and the air had left the tracheae, but on being transferred to a dish of water it hatched within 15 minutes without any rubbing movement of the head, and the air then spread into the tracheal system. The coming and going of the air in the tracheal system of the larva before it emerges is linked with the activity of the larva; when the larva ceases to move the air disappears, when it resumes movement the air may reappear. Wigglesworth (1930) finds that the activity of a newly hatched insect, by liberating metabolites and so increasing osmotic pressure, will aid the absorption of liquid, and air will extend down the tracheal trunks towards the active tissue.

9. The "Egg-bursters"

The above observations on living larvae mounted in excavated slides gives no support to the view that the frontal spines really serve to pierce the egg-shell. The darkening of the basal area on which the spines are situated, long before the final darkening of the head after emergence, would appear to indicate some metabolic activity, and it remains theoretically possible that under natural conditions the enhanced movements of the gut in the anterior part of the larva may drive the body fluid into the head and inflate the depression in which the spines lie, so that one or other of them pierces the embryonic cuticle and the egg-shell. However from watching hatchings repeatedly in a watch-glass with an ample supply of water my impression is that the split in the egg-shell appears exactly in the middle line (*i.e.* between the egg-bursters) and it seems very probable that in the soft-shelled eggs of this water beetle the egg-bursters no longer function to effect a split, but possibly they are of some use firstly, as already suggested, in cutting the embryonic cuticle, and secondly in protecting the head of the larva in its forward thrust against the egg membranes. It is interesting to find that Bertrand (1928), who has watched the eclosion of this species, states that he did not see the frontal tubercles put into use, while Blunck (1921) writes of the hatching of this *Agabus* "Ob dabei die Eizähne wirklich die ihnen gemeinhin zugeschriebene Rolle spielen, bleibt zu prüfen."

In terrestrial beetles there appears from van Emden's work to be no doubt that the egg-bursters function, for in Carabidae he has seen them in profile piercing the chorion (van Emden, 1925). Sikes and Wigglesworth (1931) state that in *Tenebrio molitor* the chorion is so fragile that no mechanism for hatching is needed. Balfour-Browne (1922) found no special hatching apparatus in the egg of *Pelobius tardus*, the shell rupturing from end to end along the ventral side of the embryo.

10. The Emerged Larva

Once out of the egg-shell the *Agabus* larva rests a few moments with its long legs draped backwards. The posterior part of the body, the legs and the cerci are usually entangled within the sloughed off remnants of the embryonic cuticle which is always brought out of the egg with the larva, and at the moment of hatching still encloses the bristles of the cerci in a long delicate sheath. This cuticle is soon pushed off with movements of the legs and is always to be found near where the larva has hatched as a crumpled exuvia, exceedingly delicate and transparent. The newly emerged larva is still pale with the gut contents yellow and with black body bristles, but usually within five hours it becomes grey, with the head dark greyish-brown.

III. NOTES ON OVIPOSITION IN OTHER SPECIES OF *Agabus*

1. *Agabus sturmi* Gyllenhal

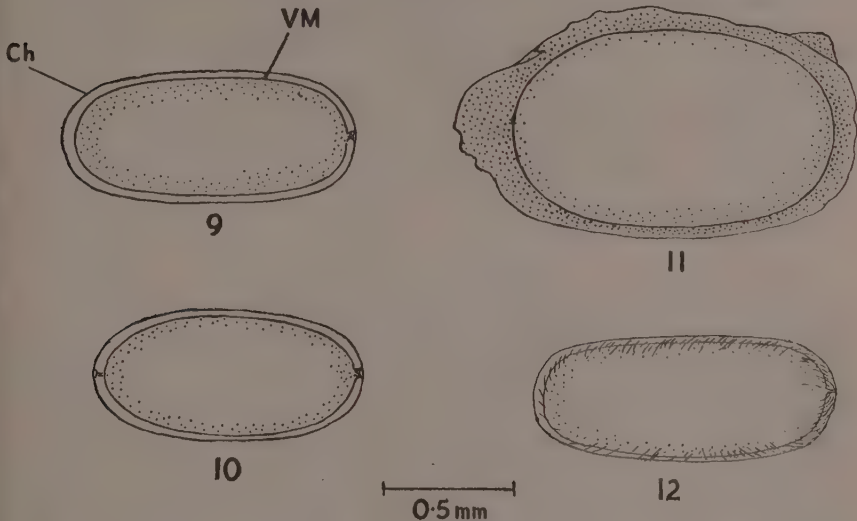
This is a common and widely distributed species in ponds in Fife, and many of the eggs of this beetle which I collected were parasitised by *Caraphractus cinctus*.

Time of oviposition.—From the dissection of specimens collected in Fife, the female reproductive organs were found to be well developed from March till the end of June, but from July until March they were immature. These observations are corroborated by observing the laying of captive specimens. Females collected in the end of September, 1955, and kept throughout the winter, did not start laying till April. Specimens collected from March till June usually laid eggs on capture, but from specimens collected in August only a single egg was obtained. Oviposition in this species would thus appear to be restricted in Fife to the spring and early summer. Soft specimens are most common in July and August but have been taken occasionally in September, October and November.

Genitalia.—The genitalia are closely like those of *A. bipustulatus*, except that the dorsal sclerotic process of the valvifer is directed towards the pygidium instead of towards the anus, and is hook-shaped. The ovipositor blades, like those of *A. bipustulatus*, are feebly sclerotic and though suited to probing amongst leaves would appear to be incapable of cutting plant tissue.

Oviposition.—The eggs of this species were found under the sheathing leaves of *Juncus articulatus* and of *J. bulbosus* L. and were often placed in rows under partially withered leaves. They occurred also on rootlets of *Juncus*, amongst the root hairs, and on withered leaves of *Carex nigra* (L.) Reichard (*caespitosa* L.) and of *Potamogeton pectinatus* L. In captivity the beetles laid readily under the sheathing leaves of *Juncus*, slightly yellowing leaves being preferred, and they also laid on sphagnum moss, fitting each egg into a leaflet as does *A. bipustulatus*.

The egg.—The egg is of closely similar appearance to that of *A. bipustulatus*, but smaller. Two measured within 24 hours of laying were respectively 1.12 by 0.52 mm., and 1.16 by 0.53 mm. The chorion fits very loosely over the vitelline membrane, so that many creases show in it (fig. 12). A micropylar area is distinct at the anterior end and it is of the same appearance as in



FIGS. 9-12.—Eggs of: (9) *Agabus labiatus* Brahm from one to six days old; (10) *A. nebulosus* Forst. from one to seven days old; (11) *A. chalconatus* Panz. surrounded by adhesive brown cement; (12) *A. sturmi* Gyll., within about 24 hours of laying, showing creases in chorion.

A. bipustulatus. The egg increases in size during embryonic development. The eggs hatched in March in an unheated room in from 14 to 15 days.

2. *Agabus nebulosus* Forster

This is a widely distributed species in Fife, occurring in ponds and other standing waters but it is rarely common.

Time of oviposition.—Specimens collected in August, 1956 and kept in an unheated room did not begin to lay till January, February and March. They continued to lay for a few weeks, but on 24th April observations were discontinued as I had obtained sufficient eggs for my experiments on breeding *Caraphractus* on host eggs of different sizes.

Genitalia.—As compared with *A. bipustulatus* the female genitalia are remarkable on account of the transparency of the eighth sternites, only the proximal and ventral areas being suffused with brown. This species is, of course, much less deeply sclerotic than *A. bipustulatus*, especially in regard to the elytra, the abdominal terga and the seventh sternum. In *A. nebulosus*, in consequence of the transparency of the eighth sternite, one can see the valvifer clearly, and its shape differs from that of *A. bipustulatus* since it is like an inverted T. The transverse bar of the T is darkly sclerotic and articulates posteriorly

with the base of the ovipositor blade, and anteriorly it forms the strut-like apodeme. The upright part of the sclerite—the shaft of the T—forms a wide straight bar which has a backward prolongation near the anus, like *A. sturmi*. The ovipositor blades are like those of *A. bipustulatus* but paler, and the vulval sclerites are faintly sclerotic.

Oviposition.—The laying habits of this species are just the same as those of *A. bipustulatus*, but I have not found the few specimens I observed nearly as prolific. The females laid on leaves of *Elodea*, under sheathing leaves of grasses, and in the hollow of the leaves of *Sphagnum* and *Hypnum*.

The egg.—The egg (fig. 10) is small for the length of the beetle but *A. nebulosus* is a flatter insect than *A. bipustulatus*. Two eggs measured within a week of laying were 1.02 by 0.54 mm. and 1.11 by 0.52 mm. respectively. The egg is just like that of *A. bipustulatus* and has a micropyle-like spot at each end (fig. 10). The eggs hatched in about three weeks.

3. *Agabus labiatus* Brahm

This small *Agabus* is very local in Fife, occurring mostly in or near moorland districts in swampy ponds or ditches, often amongst sphagnum moss.

Time of oviposition.—From specimens collected in October, 1956, and fed regularly, only one survived the winter. This specimen started to lay in the end of January and continued to lay until March, when she also died. Dissections made of *A. labiatus* collected in Fife during several years show that there is much individual variation in regard to the development of the ovaries, but mature females have been obtained in November, January and March. I have no data for Fife specimens in April, but females collected in Hampshire in that month were all mature or nearly mature. The laying period is probably from November till April.

The genitalia.—These are similar to those of *A. sturmi* but the lower part of the valvifer is more sclerotic and nearer to the shape of this sclerite in *A. nebulosus*.

The egg.—Three eggs, measured within a week of laying, varied from 1.11 by 0.53 mm. to 1.19 by 0.43 mm. (fig. 9). They are actually a trifle longer than the eggs of *A. nebulosus*, though the latter is the larger beetle.

Oviposition.—The eggs were deposited neatly in sphagnum leaves and I have no doubt that this will be the natural site. The actual duration of the incubation period was not observed but is within a month.

4. *Agabus paludosus* F.

This species, which frequents running water, is not very common in Fife. Specimens with fully developed ovaries have been obtained in Scotland in March and June, immature females in October and December. A few females were collected near St. Andrews in early October, 1956. One began to lay in February and another in March. The genitalia are similar to those of *A. sturmi* but very weakly sclerotic, and the soft ovipositor blades would be incapable of penetrating plant tissue. The egg is surrounded with a little brownish cement. It is shaped like that of *A. bipustulatus* and has a micropylar spot at both ends. The chorion lies loosely round the egg at both poles. The eggs were laid in rows under the brown sheathing leaves of grass, or placed exposed on sphagnum or other plants. None of the eggs were fertile, so that the females

had not mated before capture; probably with this species mating and laying occurs chiefly in the spring and early summer.

5. *Agabus chalconatus* Panzer

This species occurs in Fife mostly in stagnant water in moorland districts, where there is peat and sphagnum moss. All the males taken in Fife have been var. *melanocornis* Zimm. Balfour-Browne (1950) does not record the type form from Scotland.

Time of oviposition.—Though several females were collected in October, 1956, only a single egg was laid in the middle of October, but eggs were obtained from January to April. A few females from Fife and Nairn (the latter from Dr. R. Richter) have been dissected. Those killed in June and July were mostly immature, others collected in August and October had mature ovaries. These data suggest that the species lays principally from autumn till spring.

Genitalia.—The genitalia (fig. 2) present striking differences from the type found in the other species dealt with. The ovipositor blades are sharply pointed and strongly sclerotic. They are not flattened from side to side but more rounded in diameter with the point awl-shaped. They bear a subterminal tuft consisting of only a few bristles, but a thick fringe of bristles is present along the lower edge of the blade. The vulval sclerites are much more strongly sclerotic than in any of the previous species and the valvifer is greatly modified, the internal strut being very large in comparison with the rest of the sclerite, though the eighth sternite (which has been removed in fig. 2) is less sclerotic than in *A. bipustulatus*. All these features point to a powerful boring action of the ovipositor, for the wide internal strut of the valvifer will allow for a greater attachment of muscles to steady the base of the ovipositor blades during action.

Oviposition.—When no soil was provided in the tumbler with the females a few eggs were laid—anyhow—on the glass of the container or on the plants provided. They were stuck on with a good deal of brown cement, but the eggs were not fitted into sphagnum leaflets as in the other species dealt with, and none were laid under sheathing leaves of *Glyceria*. However, on adding earth or peaty soil to the tumbler, eggs were obtained. These were discovered by washing out the tumbler, when small lumps of earth were left in the bottom. Examined through the glass, an egg was found in each lump, firmly fixed to the glass and with particles of earth and sand cemented round it. Sometimes several eggs were clustered together, each with its own earthen covering. The eggs had been deposited under 2 inches of peaty soil, but the soil was loose and the beetle would be able to push its way down. I have no doubt that in ponds the females lay their eggs in the peaty soil on roots or stones, or against whatever firm surface they encounter with their ovipositors.

The egg.—The egg of *A. chalconatus* differs in many ways from the eggs of the other species described. Thus it is far larger relative to the size of the beetle and it is of a broader shape. A newly laid egg of *chalconatus* measured 1.27 by 0.65 mm., and eggs containing fully formed larvae varied from 1.45 by 1.02 mm. to 1.47 by 1.19 mm., being actually wider than the fully developed egg of *bipustulatus* (which varies from 1.65 by 0.82 mm. to 1.71 by 0.85 mm.) although *chalconatus* is a much smaller beetle than *bipustulatus*. Unlike the

translucent eggs of other *Agabus* species, the egg of *chalconatus* is opaque and of a creamy-white colour and the vitelline membrane cannot be seen through the chorion in surface view. The micropylar area with its radiating processes only shows in cleared preparations.

The opacity of the shell is due to the occurrence of minute spots of granular appearance distributed all over the surface of the chorion. When seen from above they are usually but not always spherical, and occasionally they are double. They vary in size from 2 to 7 μ . In mounted preparations of the shell in euparal or polyvinyl lactophenol, these granular bodies stand out bright and yellowish against a duller background. Mounted in water the spots usually show a double contour (fig. 13A), the outer rim being lighter; this outer circle becomes very distinct if the shell is placed in kerosene. With certain stains these granular bodies show up in a different colour from the rest of the chorion. With Mallory's orange G and aniline blue mixture they appear bright pale blue while the rest of the chorion is orange, and the vitelline membrane is blue. With acid fuchsin the chorion stains a vivid deep pink and the spots are iridescent, changing as one focuses from pale pink to pale blue, often with a white central spot. In the ovarian egg these structures show distinctly all over the chorion but at this stage the chorion is not opaque so that one can see the vitelline membrane within.

Living eggs were examined several weeks after laying, mounted in water on an excavated slide. With strong light falling on them from above, the spots on the chorion showed a silvery sheen. If the water is removed and the egg is flooded with kerosene, the silvery appearance goes and the shell becomes homogeneous and transparent, so that one can see the embryo quite clearly, and even the embryonic cuticle where it surrounds the various appendages may be distinguished. As the chorion clears, minute bubbles appear all over the surface of the egg.

Wigglesworth and Beament (1950), in discussing the respiratory mechanism of some insect eggs, find that the air in the shell is usually confined to an inner layer of porous protein, communicating with the atmosphere through pores of varying type. It seems probable that, in the egg of this water beetle, the remarkable structure of the chorion may be of respiratory significance, since, as will be shown later, the eggs of this species remain buried in the soil for many months. Professor V. B. Wigglesworth has kindly examined one of my slides of an egg of *chalconatus* and he writes that the granules appear to be flask-shaped pits and that it is possible that these pits may contain an air-filled protein.

The chorion of this egg is much stronger and more rigid than is the chorion of the eggs of the other *Agabus* described. This will serve to protect the egg whilst being laid in the soil and will probably render the egg more resistant to desiccation. It is clear that many problems regarding the egg of *chalconatus* remain to be studied and a thorough investigation of the shell would be of much interest.

Another remarkable feature of the eggs of *chalconatus* is the prolonged incubation period. A delayed hatching of autumn-laid eggs was observed by Mr. J. Balfour-Browne (see F. Balfour-Browne, 1950: 126 and 257), and my observations prove that eggs laid in winter and in spring take months to hatch. Thus, from eggs deposited in January by beetles collected in October, two larvae hatched at the end of April and one in early July, but the others

had not hatched by the middle of August. Yet the larvae within these eggs were still alive by the 22nd July for, on examining them under the microscope in a strong light, the larvae were seen to move their mandibles, six months after laying! Moreover, at the end of August a larva had its head out and was trying to emerge from an egg that had been laid in January. The apices of the antennae, mandibles and palpi become dark many weeks before the eggs

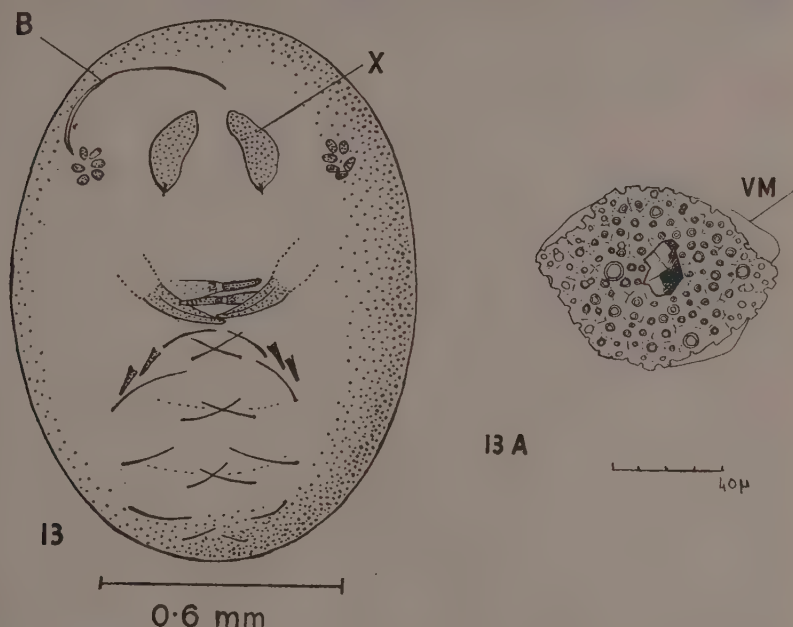


FIG.—13. *A. chalconatus* Panz. with larva almost ready to hatch. (To simplify the drawing the adhesive brown cement is not shown.) Same scale as figure 8. (A) Surface view of small area of the shell removed from an egg of *A. chalconatus*. In the centre is a piece of grit.

hatch, and also the long pigmented spot behind each egg-burster (fig. 13), but little of the rest of the larva (excepting the dark bristles) shows through the opaque shell. The larva figured in this egg (fig. 13) was drawn on 22nd April, 1957, but did not hatch till early July. On hatching, a long slit is left in the egg-shell and the embryonic cuticle is brought out of the egg as in *Agabus bipustulatus*. The eggs were kept in an unheated north room but were not exposed to frost, and possibly under natural conditions the winter-laid eggs would all hatch in spring. Not only does the larva remain for long periods fully formed within the egg, but the embryonic development is also very slow and variable. In some eggs laid in spring the ocelli of the larva, pink when first visible, did not show by the middle of July, while in other eggs of the same batch the larvae were fully formed at this time, but none of the spring-laid eggs had hatched by the middle of August.

It is probable that the long egg period and also its variable duration will contribute towards the survival of eggs with resting larvae both during periods of frost and of drought. The shallow moorland pool near St. Andrews in which I took some of the egg-laying females in March, was dried up in June and July, but the soil was damp, and, if the eggs were buried within the peat, they would have every chance of surviving till the pool again filled with water. Evidence suggesting that this had, in fact, occurred, was obtained five weeks later, on 21st August, when on revisiting the same pool I found it full of water. Larvae of *chalconatus* were very common, mostly in the first or second instar but a few in the third instar.¹

IV. DISCUSSION

It is evident that the laying habits of water beetles must be closely related to other aspects of their ecology. Owing to the scarcity of information on the bionomics of the different *Agabus* species, much still remains to be discovered, but it will be of interest to bring together here some observations bearing on ecology and oviposition. Apart from Blunck's notes on egg-laying in *Agabus undulatus* (1913a) and *A. bipustulatus* (1921), very little information has been recorded on oviposition in this large genus, which, according to Guignot (1933 : 525), numbers nearly 200 species. The present investigation, confined to six species, shows that two types of oviposition occur, five species depositing their eggs on plants under water and one species, *chalconatus*, burying its eggs in the soil. According to Blunck, *undulatus* also lays its eggs on leaves of water plants. All these species which lay on plants have soft ovipositor blades, only feebly sclerotic, which are flattened from side to side and rounded at the tip. They are adapted for probing amongst the leaves, and especially for pushing between the sheathing leaf and the stem of grasses, *Juncus*, etc., in order to place the eggs so that they lie where the leaf sheath and the stem are in contact and where moisture remains even if the water recedes from the foliage. The ovipositor of *chalconatus* is differently shaped and has strongly sclerotic pointed blades. Possibly the structure of the ovipositor may provide a clue to the method of laying. The ovipositor blades of *Agabus affinis* Payk., *A. congener* Thunb., *A. uliginosus* L., *A. arcticus* Payk., *A. undulatus* Schr. and *A. conspersus* Marsh. are similar in appearance to those of *A. bipustulatus*; in *A. guttatus* Payk. and *A. biguttatus* Ol. the blades are more elongated and sclerotic towards the apex, though not approaching the condition found in *chalconatus*.

It is evident that the manner and the time of oviposition must have an important bearing on the survival of the eggs in different types of habitats. Two of the principal dangers to which the eggs will be subjected are drought and parasitism by Chalcidoidea. In a previous paper (Jackson, 1956a) I have recorded that the parasitism of eggs, believed to be those of *Agabus sturmi*, by *Caraphractus cinctus* was very high, 69 out of 95 eggs being parasitised. The parasitism of the eggs of *Dytiscus marginalis* L. by this Mymarid, and by a Eulophid, was even higher, all 57 eggs collected in 1955 containing parasites. This year (1957) it was noticed that almost the only eggs of *Dytiscus* to escape

¹ The larvae were easily recognised as I had already bred larvae from *chalconatus* eggs. The first instar is very characteristic, and in all three instars the mandibles are denticulate. Mr. Balfour-Browne informs me that no other known *Agabus* has denticulate larval mandibles and in this character the species is unique as far as is known.

parasitism were those which had been laid very early in the year and which I collected on 12th March before the parasite was active. Further proof of the destruction wrought by *Caraphractus* amongst the eggs of water beetles during the summer was obtained in August this year. By splitting up the hollow leaves of *Juncus bulbosus* which were floating in a quarry pond near St. Andrews, I obtained just over 50 eggs typical of those of *Ilybius fuliginosus*; a few died possibly from a protozoal infection, only five developed normally (all *fuliginosus* larvae) and 46 were parasitised by the Mymarid. The fertility of *Caraphractus* is high. The offspring of a single female totalled 84, 64 females and 20 males, bred from 30 eggs of *A. bipustulatus*; moreover the newly emerged females start laying within a few hours, and I have reared five generations in the year. It is thus clear that, under favourable conditions, the Mymarid will multiply exceedingly, and the fact that individuals of various generations undergo a more or less prolonged diapause in the prepupal state within the host egg will ensure the survival of the parasite when host eggs are scarce.

It will be of interest to consider how the habits and powers of dispersal of the different species of *Agabus* may lessen the dangers from drought and parasitism. It has already been pointed out (p. 55) that the eggs of *bipustulatus* laid from late autumn till spring are unlikely to suffer from these conditions. Moreover this species and *A. nebulosus* fly readily, having powerful flight muscles, and are frequently to be found in new waters, and this habit will promote their dispersal to waters where the Mymarid may be less common. *A. sturmi*, on the other hand, while depositing its eggs in the same manner as *bipustulatus*, does so in spring and early summer when *Caraphractus* is actively laying, so it is heavily subject to parasitism. It is not a species which, in my experience, flies as readily as *bipustulatus* or *nebulosus* and I have usually found it in ponds of permanent water. *A. paludosus* frequents ditches, streams and rivers, and, although its flight muscles appear to be only rarely developed (Jackson, 1956b), it will be able to disperse along the whole drainage area of a river, and therefore likely to find sufficient water for the development of its eggs. *A. labiatus* is largely restricted to swampy moorland with permanent bogs and is accordingly very local. I have evidence to suggest that this species is flightless (Jackson, 1956c), so it will not disperse easily, but the eggs laid in sphagnum moss in the wet areas which this beetle frequents will have good chances of survival, and the winter-laid eggs will escape parasitism.

A. chalconatus is a widely distributed species to be found chiefly in moorland districts. It is a strong flier and often frequents shallow peaty pools. The eggs undergo a slow development and the larvae may remain resting within the eggs for months; this will favour survival during unfavourable conditions. Eggs buried in the soil are also little liable to be attacked by *Caraphractus cinctus*; moreover I have found that the eggs of this beetle are an unsuitable host for the Mymarid. I exposed some eggs in their earthen cells to laying females, having removed the surrounding soil. The females brought about the death of the eggs, but only in a few cases were imagines reared and these had the abdomen distended or were otherwise deformed or crippled. The eggs of *labiatus*, *nebulosus*, *bipustulatus* and *sturmi*, on the contrary, proved, in my breeding experiments, to be excellent hosts for this parasite and I have no doubt that the eggs of *paludosus* would also be suitable, being of the same type, but no fertile eggs of this species were available and I have failed to rear

Caraphractus from infertile host eggs. The habit of *chalconatus* of laying in the soil must afford a greater security, but this is probably offset by a smaller egg production. The captive females laid far fewer eggs than *bipustulatus*; in fact, so large is the mature egg of *chalconatus*, that it is doubtful if as many would have space to come to maturity at one time. Mr. Balfour-Browne writes that he has had nine eggs laid by one female in about eight hours, but a female of *bipustulatus*, as has been stated (pp. 55-56), can lay 24 eggs in five hours, and over 1700 in a year.

V. SUMMARY

Agabus bipustulatus lays principally from late autumn until spring. During a year, one female, isolated from males, laid over 1700 eggs and throughout this period nearly all the eggs continued to hatch.

The female genitalia of this species are described. The ovipositor is unsuited for cutting plant tissues.

The eggs are laid on aquatic plants, attached by a gelatinous cement, and the most favoured position is under the sheathing bases of the leaves of *Glyceria*, *Juncus* and *Carex*. The female inserts her ovipositor between the leaf sheath and the stem sliding it along as she moves up or down the stem and depositing the eggs in a row behind her.

Eggs laid under sheathing leaves are more resistant to drought, and they will have some protection against predators; the Mymarid, *Caraphractus cinctus* Walker, has some difficulty in parasitising the eggs when they are closely covered with a tough leaf sheath.

The egg of *Agabus bipustulatus* is described. The chorion, which loosely surrounds the newly laid egg, readily slips off if the egg is removed from a leaf, and in some cases an egg, deprived of its chorion and enclosed only by the vitelline membrane, continued its development, and the larva duly hatched. Under normal conditions however, contrary to the statement by Blunck, the chorion persists, but latterly it is in close contact with the vitelline membrane.

Observations are given on the appearance of the larva before hatching. The embryonic cuticle was formed a week before hatching in eggs kept in a warm room in winter. This cuticle surrounds the body, enclosing the legs, antennae and mouth parts in individual sheaths but the two cerci in a single sheath.

Hatching is accomplished by the larva suddenly raising its head. This is done with such speed that it was impossible to see whether or not the egg-bursters, situated on the frons, came into action, but observation on larvae which had only partially emerged from the egg gave no support to the view that the frontal spines pierce the egg-shell.

Notes are given on oviposition in *Agabus sturmii* Gyll., *A. nebulosus* Forst., *A. labiatus* Brahm, *A. paludosus* F., and *A. chalconatus* Panz. The first four species have eggs similar to *A. bipustulatus* but smaller, and they are laid on aquatic plants. The eggs of *A. chalconatus*, which are very large relative to the size of the beetle, are of quite different appearance from the eggs of the other species studied, being broad and opaque. They are deposited in the soil, each enclosed in an earthen cell, and the pointed ovipositor of this species will facilitate boring into the soil.

VI. EXPLANATION OF LETTERING

<i>A</i> , anus.	<i>VU</i> , position of vulva.
<i>B</i> , bristles of cerci.	<i>VS</i> , vulval sclerite.
<i>Ch</i> , chorion.	<i>VIII</i> , eighth tergum (pygidium).
<i>M</i> , cut edge of membrane connecting with eighth sternite.	<i>X</i> , pigmented area behind egg-burster.
<i>OB</i> , ovipositor blade.	<i>Y</i> , dorsal part of valvifer.
<i>V</i> , valvifer.	<i>Z</i> , internal strut from valvifer.
<i>VM</i> , vitelline membrane.	7, seventh sternum.
	8, eighth sternite.

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